unc-101, a gene required for many aspects of *Caenorhabditis elegans* development and behavior, encodes a clathrin-associated protein

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Our genetic analysis indicates that the *unc-101* gene of *Caenorhabditis elegans* is required for many aspects of development and behavior, including negative regulation of vulval differentiation. We have cloned *unc-101* and found that it encodes a homolog of the mammalian medium chains of clathrin-associated protein complexes located at the *trans*-Golgi and the plasma membrane, AP47 and AP50, respectively. Therefore, clathrin-mediated events might contribute to the negative regulation of vulval differentiation. Comparison of sequences, including a full-length sequence of a *C. elegans* AP50 homolog, reveals that UNC-101 is most closely related to AP47. Mouse AP47 and nematode UNC-101 proteins are functionally equivalent as assayed in transgenic nematodes. We have sequenced the mutant alleles of *unc-101* identified in various genetic screens and shown that all but one are deletions or nonsense mutations, suggesting that these alleles severely reduce *unc-101* function.

[Key Words: *C. elegans*; *unc-101*; vulval induction; clathrin associated protein; AP47]

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Clathrin-coated pits and vesicles originate from the plasma membrane and the *trans*-Golgi in eukaryotic cells and mediate intracellular trafficking of membrane proteins (Fig. 1A; for review see Brodsky 1988; Keen 1990; Pearse and Robinson 1990; Schmid 1992). The main protein components in the coated vesicles are clathrin and their associated protein [adaptor, or assembly, protein (AP)] complexes. Whereas clathrin triskelions are common structural units to both the plasma membrane and the *trans*-Golgi coated vesicles, the AP complexes differ in these compartments, probably conferring specificity to clathrin vesicles. The *trans*-Golgi-associated protein complex AP-1 consists of two large chains, β' and γ ; one medium chain, AP47; and one small chain, AP19 (Keen 1987; Ahle et al. 1988; Matsui and Kirchhausen 1990; Fig. 1B). The plasma membrane-associated protein complex AP-2 consists of two large chains, α and β ; one medium chain, AP50; and one small chain, AP17. Although the large chains β and β' are similar in sequence (Kirchhausen et al. 1989), the large chains α and γ have a more diverged primary structure. Both medium and small chains of the plasma membrane-associated complex are homologous to their *trans*-Golgi counterparts. Coated vesicles of the plasma membrane

are involved in the endocytosis of membrane proteins such as LDL receptor, transferrin receptor, and epidermal growth factor (EGF) receptor, whereas *trans*-Golgi coated vesicles are involved in the sorting of proteins, such as lysosomal enzymes, and are also thought to be involved in regulated secretion (e.g., Keen 1990; Pearse and Robinson 1990; Sorkin and Carpenter 1993).

In *Saccharomyces cerevisiae*, mutants deficient in a clathrin heavy chain are defective in retention of Kex2p protein in the Golgi apparatus (Seeger and Payne 1992). However, deletion of yeast small chains of the APs has little effect on cell growth, protein export, or endocytosis (H. Phan and G. Payne, pers. comm.). Similarly, deletion of a yeast medium chain does not result in any obvious phenotypic consequences, although there might be subtle phenotypes associated with these mutations (S.K. Lemmon, pers. comm.). Because yeast is a single-cell organism and yeast clathrin vesicles and their APs may have more restricted functions than those in multicellular organisms, it is useful to examine the functions of coated vesicles in a multicellular organism. Bazinet et al. (1993) have recently found that clathrin heavy chain is essential in *Drosophila*.

unc-101 mutants of *C. elegans* were originally identified by their uncoordinated movement (D. Riddle, pers. comm.) and by abnormal uptake of dye by sensory neurons (E. Hedgecock, pers. comm.). We first identified

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Figure 1. Clathrin vesicles and their associated protein complexes. (A) Coated pits and vesicles. A coated pit is composed of membrane fraction, collected membrane proteins, and clathrin triskelion cage with its associated protein complex. Coated pits are invaginated to form coated vesicles, which travel to their destinations such as lysosome and the plasma membrane (modified from Pearce and Robinson 1990) (AP) AP complex; (C) clathrin; (MP) membrane protein. (B) Schematic structure of the clathrin-associated protein complex. AP-1 complex is composed of four different peptide chains: two large chains, β , γ ; one medium chain, AP47; and one small chain, AP19. AP-2, not shown, has similar structure to that of AP-1 and is composed of two large chains, α , β ; one medium chain, AP50; and one small chain, AP17 (modified from Nakayama et al. 1991).

unc-101 alleles as extragenic suppressors of a reduction-of-function mutation of *let-23* (Aroian et al. 1990), an EGF receptor-like tyrosine kinase (G. Jongeward and P. Sternberg, in prep.). Here, we genetically analyze *unc-101* and provide evidence for its role in regulating vulval induction. We describe the molecular cloning of *unc-101* and demonstrate its functional equivalence with the mouse AP47 protein. *C. elegans* thus provides an opportunity to use molecular genetics to study clathrin-associated protein complexes in a multicellular organism.

Results

Pleiotropic effects of *unc-101* mutations

unc-101 mutations have pleiotropic effects on the behavior and development of *C. elegans*. Homozygous *unc-101* animals have uncoordinated movement. They are very sluggish, do not respond to a light touch, and tend to coil. *unc-101* animals also display abnormal uptake of the dye fluorescein-isothiocyanate (FITC) (E. Hedgecock, pers. comm.). In wild-type animals, six pairs of neurons in the amphid and two pairs of neurons in the phasmid can take up FITC (Hedgecock et al. 1985). In *unc-101* animals, only one pair of amphid neurons stains, and the phasmid neurons stain faintly if at all. *unc-101* mutant animals show irregularity in their defecation cycle (Thomas 1990). *unc-101* animals also have male tail defects such as abnormal rays and spicules (S. Emmons; H. Chamberlin; both pers. comm.). In addition, *unc-101* animals are subviable; about half of *unc-101* animals do not survive

to adulthood. These inviable animals typically arrest in late first larval (L1) stage but occasionally arrest in the L2, L3, or L4 stages. We have isolated *unc-101* mutations as suppressors of the vulvaless (Vul) phenotype of *let-23(sy1)* hemizygotes (G. Jongeward and P. Sternberg, in prep.). Whereas *unc-101* homozygotes have normal vulvae, they are defective in egg-laying, possibly resulting from their defect in late vulval morphogenesis.

Genetic analysis of *unc-101*

The *unc-101* alleles *m1*, *rh6*, *sy108*, and *sy161* had been recovered previously from independent F2 screens, as described above. Although they were recovered in screens for different phenotypes, animals homozygous for these recessive alleles display an essentially identical spectrum of phenotypes. Because F2 screens could have failed to recover null alleles of *unc-101* had the null phenotype been lethal, we performed two genetic tests to define a complete loss-of-function phenotype of *unc-101*. From these experiments we conclude that the existing *unc-101* mutations reduce *unc-101* activity severely.

We first identified a deletion of the *unc-101* locus. We recovered *sy216* by its failure to complement *unc-101(rh6)* in a noncomplementation screen of 11,000 trimethylpsoralen (TMP)-mutagenized gametes (Materials and methods). Animals homozygous for *sy216* arrest immediately after hatching without any apparent postembryonic cell divisions and live for several days before dying. This arrest phenotype is distinct from that of an-

imals homozygous for all other *unc-101* alleles, which arrest and die rapidly. Four observations suggest that *sy216* is a deletion of the entire *unc-101* locus. First, we were unable to amplify any *unc-101* genomic DNA from *sy216* homozygotes (see Materials and methods). Second, a rescuing cosmid (described below) could not rescue *sy216* despite its ability to rescue the lethality of *unc-101(sy108)*. Third, Southern analysis failed to show any polymorphism in *sy216/hln1* heterozygotes using the rescuing cosmid as probe (data not shown), indicating that the deletion in *sy216* is larger than 30 kb. Finally, J.-Y. Ho and A. Rose [pers. comm.] have found that *sy216* homozygotes are deleted for DNA on both sides of *unc-101*.

We compared the existing alleles to the deletion of the *unc-101* locus. The lethality of *unc-101(sy216)/unc-101(sy108)* trans-heterozygotes is slightly enhanced (74% inviable) with respect to *sy108/sy108* homozygotes (45% inviable). However, other phenotypes, such as suppression of the *let-23(sy1)* mutation, are not enhanced (see Materials and methods). These observations are consistent with *sy108* being a severe reduction-of-function allele.

We then screened for new alleles of *unc-101* under conditions that would allow the isolation of null alleles. About half of the progeny from homozygous *unc-101* mothers die. However, all animals of the genotype *unc-101(sy108)/unc-101(sy216)* from an *unc-101(sy108)/+* mother are viable. Therefore, the maternal copy of *unc-101(+)* is sufficient to rescue the inviability of any new loss-of-function allele in trans to the visible allele *sy108*. From a screen of 20,000 F1 ethylmethane sulfonate (EMS)-mutagenized gametes, we recovered the *sy241* allele. *sy241* homozygotes are viable and have phenotypes essentially identical to other *unc-101* homozygotes.

unc-101 is a negative regulator of the vulval induction pathway

In *C. elegans*, a signal from the gonadal anchor cell induces three of six vulval precursor cells (VPCs) to generate vulval tissue (for review, see Horvitz and Sternberg 1991). Reduction-of-function mutations in the *lin-3*, *let-23*, *sem-5*, *let-60*, or *lin-45* genes result in a Vul phenotype. Each of these genes encodes a *C. elegans* homolog of a mammalian signaling or signal transduction protein: *lin-3* encodes an EGF-like growth factor; *let-23*, an EGF-receptor homolog; *sem-5*, an SH3-SH2-SH3 adaptor; *let-60*, a Ras protein; and *lin-45*, a Raf serine/threonine kinase (for review, see Sternberg 1993). Other genes (*lin-2*, *lin-7*, and *lin-10*) are also required for vulval induction. Genetic pathway analysis suggests that *lin-3* acts via *let-23*, which acts via *sem-5*. *sem-5* acts via *let-60*, which acts via *lin-45* to stimulate vulval differentiation (for review, see Sternberg 1993). *lin-2*, *lin-7*, and *lin-10* are not absolutely required for this signaling; apparent null mutations (Ferguson and Horvitz 1985) act like partial reduction-of-function mutations of *let-23*. These three genes appear to act at the *let-23* step in the signaling pathway.

We recovered two alleles of *unc-101* (*sy108* and *sy161*) as suppressors of the Vul phenotype of the weak *let-23* allele, *sy1*. Although mutations at the *unc-101* locus confer no defect in the extent of vulval differentiation in the absence of another mutation, averaging three VPCs forming vulval tissue per animal, several non-null mutations of *let-23* are suppressed by an *unc-101* mutation (Table 1). *let-23(sy1)*, the allele used in the original screen, is suppressed strongly. *let-23(sy1)* animals average one VPC per animal forming vulval tissue, whereas *unc-101;let-23(sy1)* animals are hyper-responsive to the signal, averaging 3.6 VPCs forming vulval tissue per animal. After ablation of the gonad of the *unc-101(sy108);let-23(sy1)* double mutants, none of seven animals displayed vulval differentiation, indicating that vulval differentiation in the double mutants still requires the signal from the gonad as in *unc-101* mutant ($n=6$) or wild-type animals. A more severe mutation, *let-23(sy12)*, is suppressed from an average of less than one to nearly three VPCs per animal forming vulval tissue. Another severe allele, *let-23(sy97)*, is only slightly suppressed, if at all. The *let-23(n1045)* allele is enhanced by an *unc-101* mutation (Table 1; see Discussion).

To determine whether the interaction of *unc-101* is limited to *let-23*, we constructed a series of double mutants and examined their extent of vulval differentiation (Table 1). *unc-101* mutations suppress reduction-of-function mutations in several of the genes required for vulval differentiation. Strong reduction-of-function alleles of *lin-2*, *lin-7*, or *lin-10* cause a Vul phenotype with an average of 0.4–0.9 VPC per animal undergoing vulval differentiation. *unc-101* mutations can suppress the Vul phenotype of each of these mutants to an average of 3.4 VPCs differentiating per animal. In contrast to this strong suppression, reduction-of-function mutations of *lin-3* (the inductive signal) or *lin-45* (a downstream signal transducer) are only partially suppressed by an *unc-101* mutation. Specifically, *lin-3(n378)* homozygotes average less than one VPC per animal forming vulval tissue, whereas *unc-101;lin-3(n378)* double mutants average slightly more than two VPCs forming vulval tissue per animal. Animals bearing *lin-3(e1417)* also display this partial suppression. *lin-45(sy96)* animals average 1 VPC forming vulval tissue, whereas *unc-101;lin-45(sy96)* animals average 1.9 VPC forming vulval tissue. A dominant interfering mutation of *let-60 ras* is not suppressed. We conclude that *unc-101* is a negative regulator of vulval differentiation. A simple interpretation of the spectrum of *unc-101* suppression is that *unc-101* normally acts closer to the *let-23* step than at either upstream (*lin-3*) or downstream (*let-60*, *lin-45*) steps in this signaling pathway.

let-23 is necessary for several aspects of development (Aroian and Sternberg 1991; Aroian et al. 1993a). *unc-101* mutations suppress *let-23* mutant phenotypes in a subset of these tissues. Complete loss-of-function alleles of *let-23* confer L1 larval lethality on all homozygotes. An *unc-101* mutation does not suppress the lethality of homozygotes for complete loss-of-function alleles of *let-23* nor the incompletely penetrant lethality conferred by

Table 1. Interaction of *unc-101* with other genes in the vulval induction pathway

Genotype	<i>unc-101</i> (+)		<i>unc-101</i> (<i>sy108</i>)	
	VPCs/animal differentiating	animals with >3 VPCs differentiating (%)	VPCs/animal differentiating	animals with >3 VPCs differentiating (%)
+	3.0	0	3.0	0
<i>lin-3</i> (<i>e1417</i>)	0.8	0	1.4	0
<i>lin-3</i> (<i>n378</i>)	0.8	0	2.1	0
<i>let-23</i> (<i>sy97</i>)	0.0	0	0.06	0
<i>let-23</i> (<i>sy12</i>)	0.02	0	2.9	10
<i>let-23</i> (<i>sy1</i>)	0.8	0	3.6	40
<i>let-23</i> (<i>n1045</i>) (15°C)	1.1	0	0.2	0
<i>let-23</i> (<i>n1045</i>) (20°C)	2.5	10	0.6	0
<i>let-23</i> (<i>n1045</i>) (25°C)	3.4	40	2.0	0
<i>lin-2</i> (<i>n768</i>)	2.8	0	3.2	20
<i>lin-2</i> (<i>e1309</i>)	0.5	0	3.4	40
<i>lin-7</i> (<i>e1413</i>)	0.9	0	3.4	40
<i>lin-10</i> (<i>e1439</i>)	0.5	0	3.4	30
<i>let-60</i> (<i>sy100 dn</i>)	0.0	0	0.0	0
<i>lin-45</i> (<i>sy96</i>)	0.9	0	1.9	10

Vulval differentiation in ~20 animals per each genotype was examined using Nomarski optics. A wild-type animal has 3 VPCs induced. Fewer than 3 VPCs induced result in a Vul phenotype. When >3 VPCs generate vulval tissue, a normal vulva and pseudovulval tissue are usually produced.

the *sy97* mutation. The sterility of the allele *let-23*(*sy12*) is partially suppressed. Whereas all viable *let-23*(*sy12*) hermaphrodites are sterile, ~65% of *unc-101*;*let-23*(*sy12*) animals are at least slightly fertile. The fact that *unc-101* mutations do not suppress a complete loss-of-function mutation for this pathway suggests that *unc-101* mutations do not simply allow a bypass of this signaling pathway but, rather, relieve some negative regulation that impinges on the signaling pathway.

Molecular cloning of the *unc-101* locus

To clone *unc-101*, we correlated the genetic and physical maps around this locus by identifying and mapping transposon polymorphisms (Fig. 2). Multipoint restriction fragment length polymorphism (RFLP) mapping (Ruvkun et al. 1989) was used to map two Tc1 transposon polymorphisms, *syP6* and *syP7*, with respect to the genetic markers *unc-75*, *ced-1*, *unc-101* and *unc-59*. No recombination events were observed between *syP7* and *unc-101* in the 77 recombinants, indicating that *syP7* is very close to *unc-101*. Because the physical distance between the two polymorphisms is ~600 kb and because *syP7* is inseparable from the *unc-101*(+) marker, we tested genomic cosmid clones within 100 kb of *syP7* for their ability to complement the *unc-101* phenotype.

We identified a genomic cosmid clone that can rescue the uncoordinated phenotype of *unc-101* mutations when introduced as an extrachromosomal multicopy transgene by microinjection (Fig. 2). Only cosmid W05A3 was able to rescue *unc-101*(*sy108*). A 6.3-kb subclone of W05A3, pJL5, is the smallest genomic fragment

capable of rescuing the uncoordinated phenotype. The pJL5 subclone rescues at least three other phenotypes of *unc-101*(*sy108*): lethality, defective uptake of FITC, and suppression of *let-23*(*sy1*) Vul phenotype. The partial lethality of *unc-101*(*sy108*) was rescued in the transgenic animals carrying pJL5 from 47% to 72% survival (Table 2). pJL5 also suppresses the FITC staining defect of *unc-101*(*sy108*) animals (data not shown). The vulval differentiation of *unc-101*(*sy108*);*let-23*(*sy1*) animals carrying pJL5 as a transgene was lowered from 3.6 VPCs induced per animal, as seen in *unc-101*(*sy108*);*let-23*(*sy1*) double mutants lacking the transgene, to 0.8 VPC induced per animal (*n*=20), an extent similar to that of *let-23*(*sy1*) single mutants. Therefore, pJL5 also rescues the suppression of the *let-23*(*sy1*) mutation caused by *unc-101*(*sy108*). We did not test other phenotypes associated with *unc-101* mutations for rescue.

Genomic and cDNA structure of *unc-101*

We isolated a full-length cDNA clone from a cDNA library (Barstead and Waterston 1989) using the pJL2 plasmid as probe (Fig. 2) and determined its nucleotide sequence (Fig. 3A). There are three in-frame stop codons 5' to the putative ATG codon. Also, the 5' end of the cDNA sequence has 7 nucleotides that are identical to the 3' end sequence of the *trans*-spliced leader sequence SL1 (Krause and Hirsh 1987), suggesting that this cDNA has a full-length coding sequence and that *unc-101* is a *trans*-spliced gene. A polyadenylation signal, AATAAA, is present at nucleotide 1844. The nucleotide sequence predicts that the *unc-101* gene product is a protein of 422 amino acids.

We inferred the genomic structure of *unc-101* by partial genomic sequence data and PCR with primers specific to cDNA regions (Fig. 3B). The *unc-101* cDNA is divided into seven exons. The smallest rescuing plasmid, pJL5, has 95 nucleotides 5' to the SL1 acceptor sequence.

Sequences of *unc-101* mutant alleles

To confirm our identification of the *unc-101*-coding region, we analyzed the lesions associated with *unc-101* alleles. We determined the locations of the mutations of

eight alleles of *unc-101* (Fig. 3B). We determined the mutations by directly sequencing PCR-amplified DNA preparations from genomic DNA or single mutant animals [Kretz et al. 1989]. All but one allele are predicted to produce truncated proteins due to either a deletion or a nonsense mutation. *sy108* is a deletion of 115 nucleotides in exon 3 and intron 3 and an insertion of 8 nucleotides at the deletion point. *sy237*, *sy241*, *m1*, and *rh6* are nonsense mutations, encoding truncated proteins. *sy237* is a G → A transition at nucleotide 550, changing a TGG codon to a TAG stop codon. *sy241* is a C → T transition mutation at nucleotide 1284, changing a CAA codon to a TAA stop codon. *m1* is a C → T mutation at nucleotide 1314, changing a CAA codon to a TAA stop codon. *rh6* is another C → T mutation at nucleotide 1086, changing CAA to TAA. *sy161*, the only missense mutation, is a C → T mutation at nucleotide 552, changing CGC (arginine) to TGC (cysteine). This arginine residue is conserved in both AP47 and AP50 (see below; Fig. 4A). We were unable to amplify by PCR any genomic DNA from *sy216* homozygotes, suggesting that this mutation is a deletion of the entire gene (see Materials and methods).

unc-101 encodes a clathrin-associated protein

The predicted translation product of the *unc-101* cDNA sequence is highly similar to two mammalian proteins, mouse AP47, the medium chain of the trans-Golgi-associated, clathrin-associated complex AP-1, and rat AP50, the medium chain of plasma membrane-associated,

Figure 3. cDNA sequence and genomic structure of *unc-101*. (A) *unc-101* cDNA sequence and its predicted translation product. The region identical to the 3' end of SL1 sequence is underlined. The start and stop codons, and a polyadenylation signal sequence are also underlined. The sites for the construction of the mammalian AP47 hybrid gene are underlined with arrows. The first nucleotide, G, is the last nucleotide of the vector. (B) Genomic structure of *unc-101*. *unc-101* has seven exons and six introns. The size of introns varies from 50 bp to 1.0 kb. The numbers above the structures represent the first nucleotide of the cDNA after SL1 sequence, the translation starting point, and the last nucleotides of exons. The locations and characteristics of mutations are marked as such.

Figure 4. UNC-101 is a clathrin-associated protein. (A) Amino acid sequence alignment of homologs of UNC-101. AP47 and AP50 are the medium chains of *trans*-Golgi clathrin-associated protein complex and plasma membrane clathrin-associated protein complex, respectively (Thurieu et al. 1988; Nakayama et al. 1991). CEAP50 is an AP50 homolog in *C. elegans* (this study). This alignment only highlights the residues that are identical between UNC-101 and AP47 or between all four homologs. The GenBank accession numbers are L26290 for CEAP50 and L26291 for UNC-101. (B) Diagram of the comparison of identity among the homologs of medium chains of clathrin-associated complexes. The numbers represent the identity at amino acid sequence level. The most prominent homologies are marked by boldface lines. UNC-101 and AP47 are more similar to each other than to AP50 and CEAP50.

clathrin-associated complex AP-2 (Fig. 4; Thurieu et al. 1988; Nakayama et al. 1991) UNC-101 is also similar to a yeast protein, Yap54, the yeast homolog of AP47 (Nakayama et al. 1991). UNC-101 protein is 74% identical to AP47 and 42% identical to AP50, suggesting that UNC-101 is a homolog of the mammalian AP47.

To test whether UNC-101 is a homolog of AP47, we sought to identify AP50 homologs in *C. elegans*. The *C. elegans* genome sequencing consortium has identified a cDNA encoding a homolog of AP50 (Waterston et al. 1992). Using this cDNA clone as probe, we isolated three more cDNA clones and determined their sequences. All three were products of a single gene. One of the three cDNA clones had a presumably full-length coding sequence, based on homology with other medium chains. The comparison of the amino acid sequence of this predicted protein with those of other homologs revealed that it is 81% identical to AP50 and 40–42% identical to AP47 homologs, indicating that this sequence is an AP50 homolog (Fig. 4). On the basis of the extent of the amino acid sequence identity, we believe that *unc-101* encodes a *C. elegans* homolog of AP47 protein.

We physically mapped the AP50 homolog to the X chromosome by yeast artificial chromosome (YAC) grid hybridization and identified a cosmid (T16D11) that contains the coding region of AP50 homolog in the middle of its insert (data not shown). When injected into *unc-101(sy108)* animals, this cosmid did not rescue the Unc phenotype of *unc-101(sy108)* animals. This negative result is consistent with *unc-101* being a homolog of AP47.

The function of AP47 is conserved in mammals and nematodes

Because the amino acid sequence of *unc-101* is similar to that of the mammalian homolog AP47, it is conceivable that their function has been conserved during evolution. To test this hypothesis, we examined whether the mammalian AP47 gene can rescue the phenotype of *unc-101* animals. We constructed a hybrid gene with *unc-101* genomic DNA and mouse AP47 cDNA (Fig. 5). In the mouse/nematode hybrid construct, 298-amino-acid residues of 422 amino acids are from the mouse cDNA. We also constructed an *unc-101* cDNA hybrid gene that is identical to the AP47 hybrid except that it has *unc-101* cDNA instead of AP47 cDNA. As a control, we constructed an *unc-101* gene deleted for the 298 amino acids replaced by the cDNAs. Both hybrid genes rescue the Unc phenotype of *unc-101(sy108)* animals (Fig. 6); the control construct does not. The extent of vulval differentiation of the transgenic *unc-101(sy108);let-23(sy1)* animals carrying the AP47 hybrid gene was an average of 2.0 VPCs per animal ($n = 10$), compared with 3.6 VPCs of *unc-101(sy108);let-23(sy1)* animals without the transgene ($n = 20$), indicating that the suppression of the *let-23(sy1)* Vul phenotype was also rescued (Fig. 7). Thus, mouse AP47 and *C. elegans* UNC-101 are not only similar in sequence but also functionally interchangeable. Rescue of the lethality associated with *unc-101(sy108)* animals was not checked because of low transmission of the transgene.

Figure 5. Schematic of mouse/nematode hybrid gene construction. The *NruI*–*EcoRV* fragment of the rescuing plasmid pJL2 was replaced with the corresponding cDNA fragments from *unc-101* cDNA clone or mouse AP47 cDNA clone in the *unc-101* hybrid gene and in the *unc-101/AP47* hybrid gene, respectively.

Discussion

We have analyzed the *C. elegans unc-101* gene genetically and molecularly. *unc-101* mutations not only cause a variety of behavioral and developmental defects but also suppress the Vul phenotype of weak alleles of *let-23*, a *C. elegans* EGF receptor homolog required for vulval induction. We have cloned the *unc-101* gene and have shown that it encodes a homolog of AP47, the medium chain of the *trans*-Golgi clathrin-associated complex. We also determined the cDNA sequence of a homolog of AP50, the medium chain of plasma membrane-associated complex. Sequence comparison clearly indicates that UNC-101 is an AP47 homolog. This homology was confirmed by the fact that a nematode *unc-101*/mouse AP47 hybrid gene could functionally replace the nematode *unc-101* gene. *unc-101* provides the only known case in which mutations of clathrin AP genes cause visible phenotypes. Because *unc-101* mutations have pleiotropic phenotypes and because mammalian AP47 and nematode UNC-101 are functionally interchangeable, one can study the function of clathrin-coated vesicles in the regulation of signal transduction. Further study of *unc-101* and homologs of other components of the clathrin-coated vesicles on the plasma membrane and the *trans*-Golgi compartment will help us to understand the nature of the coated vesicles and their roles in a well-characterized signal transduction pathway.

Null phenotype of *unc-101*

We analyzed alleles of *unc-101* recovered in several screens to define the loss-of-function phenotype of this locus. We are convinced that the visible alleles, exemplified by *sy108* severely reduce and possibly eliminate *unc-101* function for the following reasons: (1) *sy108* in *trans* to a deletion of the locus *sy216* has a similar phenotype to *sy108* homozygotes; (2) in a screen that should be able to recover null alleles of *unc-101*, we obtained an allele with identical phenotypes to the existing alleles rather than homozygous lethal allele, for example, and

(3) all but one allele are deletions or nonsense mutations, encoding truncated and probably nonfunctional proteins.

The subviability of *unc-101* mutant animals could be explained by residual function of *unc-101* by the amino-terminal fragments. However, multicopy transgenes carrying just the amino terminus of the protein do not rescue *unc-101* mutants, although we cannot exclude readthrough of stop codons. Another possibility is general sickness caused by the cumulative effects of many *unc-101* defects. It is also possible that there is a partially redundant homolog of AP47. Our preliminary observations suggest that there is another homolog of AP47 in *C. elegans* (J. Lee and P. Sternberg, unpubl.), but whether this protein is expressed in the same cells as UNC-101 is yet to be determined.

Structure and function of AP50/AP47 proteins

It is not surprising that AP50 and AP47 sequences are similar (40% identical over 422 amino acids; Nakayama et al. 1991), because other components of the clathrin-associated complexes also are similar. Mammalian small chains AP17 and AP19 are 44% identical, and large chains β and β' , show the highest degree of identity of 84% (Kirchhausen et al. 1991). The exception is α and γ large chains, which are only 29% identical. Given the extent of similarity between the components of the APs, it is conceivable that β and β' chains have common functions, such as binding to clathrin trimers, and that the medium and small chains have some unique and some common functions. In contrast, α and γ large chains may have unique functions such as binding to particular membrane proteins.

Amino acid residues conserved in both AP47 and AP50 might have common functions such as interaction with clathrin trimers and membrane components. These residues are distributed throughout the peptide sequence. Amino acid residues specifically conserved in either AP47 or AP50 homologs may be important for their unique functions, such as interaction with particular

Figure 6. Rescue of Unc phenotype by the mouse/nematode hybrid gene. The tracks that the worms create by moving on the bacterial lawn are shown. (A) N2 wild type, 5 min after transfer; (B) *unc-101(sy108)*, 40 min after transfer; (C) transgenic *unc-101(sy108)* carrying the AP47 hybrid as a transgene, 5 min after transfer. The uncoordinated movement of *unc-101(sy108)* is suppressed by the transgene.

membrane proteins, or other components of their own type of AP complex. These residues are also distributed throughout the peptide sequence, making it difficult to predict the domain structure of the proteins. So far, we have not found residues important for AP47-specific function. *sy161* is the only missense mutation, and it

occurs at an amino acid conserved in both the AP47 and AP50 proteins. The *m1* and *rh6* alleles encode proteins missing just a few carboxy-terminal residues but confer identical phenotypes to other alleles, suggesting that the carboxy-terminal residues are important for function, stability, or regulation.

Negative regulation of *C. elegans* vulval induction

We have provided evidence that *unc-101* also acts as a negative regulator of vulval differentiation. Mutations of *unc-101* strongly suppress defects associated with mutations in genes such as *let-23*, *lin-2*, *lin-7*, and *lin-10* that are required for vulval differentiation, suggesting that one wild-type function of *unc-101* is to negatively regulate the process of vulval induction. The loss of *unc-101* activity in an otherwise wild-type animal confers no defect in the extent of vulval differentiation, suggesting that *unc-101* acts to modulate the inductive signaling pathway, rather than acting as a target of the signal transduction pathway. Consistent with this, *unc-101* mutations are not capable of bypassing the complete lack of either the inductive signal, *let-23*, or *let-60 ras*, suggesting that an *unc-101* mutation (and therefore the removal of one pathway of negative regulation) is not sufficient to promote vulval fates in the absence of inductive signal. The enhancement of the *let-23(n1045)* allele is difficult to interpret because the *n1045* mutation results in a variety of abnormal mRNAs (Aroian et al. 1993b). We suspect that some species of proteins produced might have an inhibitory negative effect on the signal transduction (see Aroian and Sternberg 1991) and that this effect is enhanced by the lack of negative regulation by *unc-101*. A defect in *unc-101*, in combination with a mutation in the *sl-1* locus, results in excessive vulval differentiation (G. Jongeward and P. Sternberg, in prep.), supporting our hypothesis that *unc-101* normally prevents excessive vulval differentiation.

Roles of clathrin-associated complexes and *unc-101*

AP complexes are thought to drive clathrin coat formation and to couple the clathrin lattice with distinct membrane proteins (Keen 1990; Pearse and Robinson 1990; Schmid 1992). The core of the complex (Fig. 1B), which contains amino-terminal halves of the large chains as well as the medium and small chains, can bind to clathrin trimers (Matsui and Kirchhausen 1990; Peeler et al. 1993). The function of each component of the AP complex is not well understood. It is possible that the medium chains function as regulators of the clathrin-associated protein complexes.

Because *unc-101* mutations have many different effects on the behavior and development of *C. elegans*, the wild-type UNC-101 protein has important functions in many different types of cells. How would UNC-101 function in these cells? Besides a general role in regulating membrane trafficking, one function could be negative regulation of receptor-mediated signal transduction.

Figure 7. Rescue of suppression of the Vul phenotype of *let-23(sy1)* by the mouse/nematode hybrid gene. The VPCs in the L3 molt stage, when the VPCs should have divided twice to generate four progeny, are shown. The triangles represent the anchor cell. (A) N2, three VPCs each generated four progeny; (B) *let-23(sy1)*, no VPC divided to generate four progeny; (C) *unc-101(sy108)*, same as N2; (D) *unc-101(sy108);let-23(sy1)*, four VPCs divided to generate four progeny; (E) *unc-101(sy108);let-23(sy1);Ex[AP47 hybrid]*, no VPC divided to generate four progeny. Bar, 20 μ m.

Further genetic analysis may help to reveal the full spectrum of *unc-101* interactions with other receptors.

On the basis of results from the biochemical studies on coated vesicles and assuming that UNC-101 is a *trans*-Golgi clathrin-associated protein, there are many possible molecular mechanisms by which UNC-101 could act within the cells involved in the *C. elegans* vulval induction. UNC-101 might regulate secretion of the LIN-3 signal within the anchor cell: UNC-101 might be involved in negative regulation of LIN-3 signal production by sequestering LIN-3 proteins from the secretion route after their synthesis. We do not favor this model because *unc-101* mutants in a *let-23(+)* background do not have any excessive vulval differentiation while overexpression of *lin-3* causes excessive vulval differentiation (Hill and Sternberg 1992). Another possible role of UNC-101 is regulation of transport of LET-23 receptors after their biosynthesis: UNC-101 might regulate LET-23 receptors by maintaining an intracellular pool of the receptors after their synthesis. This action could provide a post-translational regulation of protein activity. Hence, in the absence of an intracellular pool of the receptors, more LET-23 receptors whose activity is reduced by a reduction-of-function mutation could reach the cell surface, being capable of transducing more sig-

nal. Sorkin and Carpenter (1993) have recently shown that α -adaptins of the plasma membrane (AP-2 complex) interact with EGF receptor in A431 cells; AP-1 complexes containing UNC-101/AP47 might play an analogous role at other stages in intracellular trafficking. A third possible role of UNC-101 is attenuation of signal transduction after activation of the molecules in the pathway by directing intracellular degradation: One of the best known functions of the coated vesicles on the *trans*-Golgi is sorting of the lysosomal enzymes. This is accomplished by sorting mannose-6-phosphate receptors, which recognize lysosomal enzymes tagged with mannose-6-phosphate, and transferring these receptor/ligand complexes to the lysosome. UNC-101 might be involved indirectly in the down-regulation of signal transduction components through controlling lysosomal or prelysosomal compartments; for example, in the absence of normal compartments, receptor might be recycled inappropriately.

Materials and methods

Strains and genetic methods

Methods for culturing, handling, and genetics of *C. elegans* were described by Brenner (1974). All genetic experiments unless oth-

erwise indicated were performed at 20°C. Mutagenesis protocol using TMP was provided by M. Yandell and L. Edgar (pers. comm.). The standard strain N2 and *dpy-5(e61)* were from Brenner (1974). The alleles for examining genetic interactions of *unc-101* were as follows: *lin-3: e1417, n378* (Horvitz and Sulston 1980); *let-23: sy1, sy97, sy12* (Aroian and Sternberg 1991), *n1045* (Ferguson and Horvitz 1985); *lin-2: n768* (Ferguson and Horvitz 1985), *e1309* (Horvitz and Sulston 1980); *lin-7: e1413* (Ferguson and Horvitz 1985); *lin-10: e1439* (Ferguson and Horvitz 1985); *let-60: sy100dn* (Han et al. 1990); *lin-45: sy96* (Han et al. 1993). The starting strain for identifying the polymorphisms associated with *unc-101*, MT3618 [*unc-75(e950) ced-1(n1506) unc-59(e261)*], which has a portion of chromosome I (between *unc-75* and *unc-59*) from the TR679 mutator strain, was provided by S. Glass, T. Gerber, and R. Horvitz (pers. comm.). The *unc-101* alleles sequenced were *m1* [D. Riddle, pers. comm.], *rh6* [E. Hedgecock, pers. comm.], *sy108*, *sy161* [G. Jongeward and P. Sternberg, in prep.], *sy241* (this study), and *sy237* [J. Lee, unpubl.].

Isolation of new alleles of *unc-101*

sy216 was isolated among the progeny of TMP-mutagenized *ced-1* males mated with *unc-101(rh6);dpy-5* hermaphrodites. A rare *Unc-101* non-*Dpy* hermaphrodite was obtained after screening 11,000 gametes. A similar screen of 15,000 gametes using EMS was carried out, and two alleles, *sy168* and *sy169*, were recovered. Sequencing these alleles indicated that these are the same mutation as *sy108*, presumably the result of gene conversion or the recovery of the maternal allele.

sy241 was isolated from a screen of 20,000 progeny of EMS-mutagenized *unc-101(sy108) + eDf24/hIn1[+unc-54(h1040) +]* heterozygotes. *eDf24* is a deficiency that deletes the rRNA genes on chromosome I, conferring lethality to the homozygotes for this deficiency. *hIn1* is an inversion of chromosome I that suppresses recombination in the region between *unc-75* and *unc-54*; one version of this balancer is marked with *unc-54*, facilitating the discrimination of the homozygotes. The heterozygous self-progeny of mutagenized parents were screened for rare *Unc-101* animals, which would be *unc-101(sy108) + eDf24/hIn1[sy241 unc-54(h1040) +]*.

Correlating genetic and physical maps

The source of the *syP6* and *syP7* RFLPs was the strain MT3618 (*unc-75 ced-1 unc-59*), which contains a transposon-rich region between *unc-75* and *unc-59* [S. Glass, T. Gerber and R. Horvitz, pers. comm.], relative to the *unc-101(sy108)* strain, a typical Bristol strain. We picked 77 *Unc-59* non-*Unc-75* recombinants from heterozygous progeny of *++ sy108/+unc-75 ced-1 + unc-59* and selected animals homozygous for the recombinant chromosome. We digested genomic DNAs of two of these recombinants with *HindIII*, diluted, and self-ligated them, then used them as templates for PCR with *Tc1* internal primers of Hill and Sternberg (1992), performing the inverse PCR as described by Ochman et al. (1988). We identified two polymorphisms, *syP6* and *syP7*, 1.1 kb and 0.8 kb long, respectively, on an analytic agarose gel. We found that *syP6* was present in the congenic strain but was not present either in the *++ +unc-59* or the *++unc-101 unc-59* recombinants, indicating that this polymorphism is to the left of *unc-101* on the genetic map. *syP7* was present in *++ +unc-59*, but not in *++unc-101 unc-59* recombinants; therefore, *syP7* is near *unc-101* and to the right of *syP6*. We used the flanking sequences of *syP6* and *syP7* as probe for physical and/or genetic mapping (Williams et al. 1992). To genetically map *syP7* more accurately, we analyzed the remain-

ing 75 recombinants and performed either Southern hybridization with *syP7* as probe or PCR with *syP7* primers. For PCR, two primers were made from the region flanking the *syP7* polymorphism. EPR1, 5'-GGTGATAGCACCATATGGTTCC-3'; EPR2, 5'-ATATA GTGCTGTGCGGAAGTC-3'. We designed these primers so that if the recombinants had the polymorphic transposon, the PCR-amplified band using either of these primers and the *Tc1* internal primer would be 125 bp; long, and if not, an 80-bp band would be amplified from EPR1 and EPR2 as extending primers.

By hybridization to a YAC grid filter [Coulson et al. 1991], we located *syP6* on the physical map on the right arm of chromosome I. *syP7* was located ~600 kb to the right of *syP6* on the same set of overlapping genomic clones. Using 38 of the recombinants for which we scored the *ced-1* marker, 5 were between *unc-75* and *ced-1*, 12 were between *ced-1* and *unc-101*, and 21 were between *unc-101* and *unc-59*.

Cosmids and *C. elegans* physical map

We obtained all cosmids and the physical map data from A. Coulson and J. Sulston (Medical Research Council, Cambridge, UK; Coulson et al. 1986, 1988).

DNA-mediated transformation of *unc-101* mutants

Microinjection of cosmids or subcloned DNAs was described by Mello et al. (1991). We used *unc-101(sy108)* animals as host strain for rescuing the *Unc* and the lethality of the visible allele. We coinjected *unc-101(sy108)* animals with pRF4, which bears a dominant *rol-6* mutation that results in a rolling phenotype. Selection of this marker phenotype facilitates selecting the transgenic animals. We used *unc-101(sy108);let-23(sy1)* animals to examine the rescue of the suppression of the *let-23(sy1)* Vul phenotype. We injected these animals with the rescuing subclone pJL5 without any other marker, because the rescue of the *Unc* phenotype itself serves as a good marker for the presence of the transgene.

DNA manipulations, sequencing, and analysis

All procedures for handling genomic DNA and cDNA were as described [Sambrook et al. 1989]. The Macvector software package of IBI (New Haven CT.) and the software of the Genetics Computer Group (Program manual for the GCG package, v. 7, 1991, University of Wisconsin, Madison) was used to edit the genomic and cDNA sequences. Data base search was performed using the BLAST program. Pileup and Prettyplot commands were used to generate the comparison of the amino acid sequences.

Sequence of mutant alleles

We made 10 PCR primers from the intron sequences, 5' end and 3'-nontranslated region as follows: INT1, TTCCGCTAATTT-TCTCCGG; INT2, ATGCGTCATTTTCAACGG; INT3, CGCTCCAATGATAAAACAC; INT4, GCATTTTCGCAT-TGGAGCG; INT5, AAATGTGTTTTTCGACTCG; INT6, AAAAAGTACGCCACATCAC; INT7, AAGTCAGGCCAT-GCCTCAA; INT8, CATAAATCTCACATTGGGCA; INT9, GAGATTATGTGATTTTTTG; and INT10, CTCGGCCACGGTCTGTTTTT. With these PCR primers, we could amplify all the exons and their flanking intron sequences. To amplify by PCR the exons and their boundaries, we used genomic DNA or single worms of the homozygous mutant. We directly se-

quenced the amplified DNAs using the same sets of primers (Kretz et al. 1989).

Construction of hybrid genes

To construct an *unc-101*/AP47 hybrid gene, we used the *unc-101* rescuing plasmid pJL2, the *unc-101* cDNA subclone, and the mouse AP47 cDNA subclone as sources for the sequences. We kept the first two introns of the rescuing pJL2 plasmid in this construction because pJL2 has only 95 nucleotides 5' to the SL-1 acceptor sequence and because expression of transgenes is more efficient in the presence of introns (e.g., Brinster et al. 1988; Buchman and Berg 1988; Fire et al. 1990). The rescuing plasmid pJL2 was digested with *Nru*I and partially with *Eco*RV, and the 7.2-kb fragment was purified from a low melting point agarose gel and ligated with the 0.9-kb *Nru*I–*Eco*RV fragment from the *unc-101* cDNA subclone. The resulting plasmid was the *unc-101* cDNA hybrid gene. We made two PCR primers from the AP47 cDNA sequence to amplify the corresponding region of the *Nru*I–*Eco*RV fragment of *unc-101* cDNA: 47-1, 5'-CGACAACCTTTGTCATCATCTA; 47-2, 5'-ATCCACTCTCTCAATGATTTTC-3'. To facilitate subcloning, we replaced three most 5' nucleotides of 47-1 and 47-2 primers with the recognition sequence of *Nru*I and *Eco*RV, respectively. This replacement does not change the encoded amino acid. We ligated the amplified DNA into the pJL2 *Nru*I–*Eco*RV fragment. We confirmed the correct reading frame by regeneration of the *Nru*I and *Eco*RV sites. This construct has the 5' region of *unc-101*, including the 5' region of the rescuing plasmid pJL2 and the coding region with two introns up to cDNA nucleotide 388, fused in-frame to the AP47 cDNA from 389 to 1281, *unc-101* cDNA nucleotide from 1282 to the stop codon, and all of the 3'-untranslated region of *unc-101*. The control construct has a deletion of the *Nru*I–*Eco*RV fragment (equivalent to nucleotides 389–1281 of the cDNA).

Assay for the rescue of the phenotypes of *unc-101* animals by the nematode/mouse hybrid gene

We obtained stable lines of transgenic animals after microinjection of the hybrid gene. For the *Unc* phenotype rescue, we transferred five transgenic animals to a spot on new plates; and after the given time, we photographed the tracks that the animals created by moving on the bacterial lawn viewed in a Wild M5A stereomicroscope. For the rescue of the suppression of *let-23(sy1)* Vul phenotype, we examined the vulval induction of the transgenic animals bearing the hybrid gene in their late L3 stages using Nomarski optics.

Analysis of *sy216*

To phenotypically examine *sy216* and other *unc-101* alleles, we constructed *trans*-heterozygous animals bearing *sy216/sy108*. More *sy216/sy108* heterozygous animals (74%) are inviable than of *sy108/sy108* animals (45%). Suppression of the Vul phenotype of *let-23(sy1)* is not enhanced. *sy216/sy108;let-23(sy1)* animals averaged 3.6 VPCs undergoing vulval differentiation, which is the same level of vulval differentiation of *sy108/sy108;let-23(sy1)* animals ($n=20$ for both).

Because TMP is known to induce small deletions at high frequency (L. Edgar, pers. comm.), we suspected that *sy216* could be a small deficiency that deletes neighboring genes as well as *unc-101*. To address this issue genetically, we tested whether any of the nearest genes are deleted by *sy216*. We constructed the *trans*-heterozygote *unc-101(sy216)/eDf3*. *eDf3* fails to complement the mutations that define the nearest ge-

netically defined loci to the right: *unc-59*, *let-201*, *let-202*, and *let-203*. This heterozygote is viable and wild type. Therefore, *sy216* does not delete the nearest genetically defined loci to the right. *sy216* does not delete the nearest known gene to the left, *ced-1*, because a *sy216* chromosome can donate a wild-type copy of *ced-1* in a recombination event. Specifically, an animal of genotype *dpy-5 + sy216/hIn1* was found among the cross-progeny of a + *ced-1 sy216/dpy-5 + unc-101(rh6)* hermaphrodite mated with *hIn1* males. However, it is likely that there are essential genes in the interval between *ced-1* and *eDf3*, as this region is not saturated for lethal mutations.

To examine the rescue of the lethality of *sy216* by the rescuing cosmid W05A3, we used *sy216/hIn1 unc-54(h1040)* heterozygotes as host for the transgene. *hIn1* is an inversion that suppresses recombination in the region between *unc-75* and *unc-59* (Zetka and Rose 1992). We coinjected the W05A3 cosmid and the pRF4 marker DNAs, picked rolling F1 transgenic animals, transferred 20 F2 individual animals to new plates, and checked their segregation of *unc-54* marker. All 20 segregated *unc-54*, indicating that there was no animal of genotype *sy216/sy216* carrying a W05A3 transgene.

To amplify genomic DNA from the *sy216/sy216*-arrested animals, we picked two or three arrested animals and performed PCR as described above. As an internal positive control, we used a set of *let-23* PCR primers in the same PCR reactions with *unc-101* primers. We tried five different sets of *unc-101* primers but only recovered an amplified band of *let-23* genomic DNA from the *let-23* primers.

To detect any polymorphisms linked to *sy216*, we used genomic DNA of *sy216/hIn1* heterozygotes digested with various restriction enzymes in a Southern hybridization with W05A3 cosmid as probe. We did not detect any polymorphism compared with control *hIn1* homozygotes.

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References

- Ahle, S., A. Mann, U. Eichelsbacher, and E. Ungewickell. 1988. Structural relationships between clathrin assembly proteins from the Golgi and the plasma membrane. *EMBO J.* 7: 919–929.
- Aroian, R.V. and P.W. Sternberg. 1991. Multiple functions of

- let-23*, a *C. elegans* receptor tyrosine kinase gene required for vulval induction. *Genetics* **128**: 251–267.
- Aroian, R.V., M. Koga, J.E. Mendel, Y. Ohshima, and P.W. Sternberg. 1990. The *let-23* gene necessary for *Caenorhabditis elegans* vulval induction encodes a tyrosine kinase of the EGF receptor subfamily. *Nature* **348**: 693–699.
- Aroian, R.V., G.M. Lesa, and P.W. Sternberg. 1993a. Mutations in the *Caenorhabditis elegans let-23* EGF receptor-like gene define elements important for cell-type specificity and function. *EMBO J.* **12**(13): (in press).
- Aroian, R.V., A.D. Levy, M. Koga, Y. Ohshima, J.M. Kramer, and P.W. Sternberg. 1993b. Splicing in *Caenorhabditis elegans* does not require an AG at the 3' splice acceptor site. *Mol. Cell. Biol.* **13**: 626–637.
- Barstead, R.J. and R.H. Waterston. 1989. The basal component of the nematode dense-body is vinculin. *J. Biol. Chem.* **264**: 10177–10185.
- Bazinnet, C., A.L. Katzen, M. Morgan, A.P. Mahowald, and S.K. Lemmon. 1993. The *Drosophila* clathrin heavy chain gene: Clathrin function is essential in a multicellular organism. *Genetics* **134**: 1119–1134.
- Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* **77**: 71–94.
- Brinster, R.L., J.M. Allen, R.R. Behringer, R.E. Gelinas, and R.D. Palmiter. 1988. Introns increase transcriptional efficiency in transgenic mice. *Proc. Natl. Acad. Sci.* **85**: 836–840.
- Brodsky, F.M. 1988. Living with clathrin: Its role in intracellular membrane traffic. *Science* **242**: 1396–1402.
- Buchman, A.R. and P. Berg. 1988. Comparison of intron-dependent and intron-independent gene expression. *Mol. Cell. Biol.* **8**: 4395–4405.
- Coulson, A.R., J. Sulston, S. Brenner, and J. Karn. 1986. Toward a physical map of the genome of the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci.* **83**: 7821–7825.
- Coulson, A., R. Waterston, J. Kiff, J. Sulston, and Y. Kohara. 1988. Genome linking with yeast artificial chromosomes. *Nature* **335**: 184–186.
- Coulson, A., Y. Kozono, B. Lutterbach, R. Shownkeen, J. Sulston, and R. Waterston. 1991. YACs and the *C. elegans* genome. *BioEssays* **13**: 413–417.
- Ferguson, E. and H.R. Horvitz. 1985. Identification and characterization of 22 genes that affect the vulval cell lineages of *Caenorhabditis elegans*. *Genetics* **110**: 17–72.
- Fire, A., S. White-Harrison, and D. Dixon. 1990. A modular set of *lacZ* fusion vectors for studying gene expression in *Caenorhabditis elegans*. *Gene* **93**: 189–198.
- Han, M., R. Aroian, and P.W. Sternberg. 1990. The *let-60* locus controls the switch between vulval and non-vulval cell types in *C. elegans*. *Genetics* **126**: 899–913.
- Han, M., A. Golden, Y. Han, and P.W. Sternberg. 1993. *C. elegans lin-45 raf* gene participates in *let-60 ras* stimulated vulval differentiation. *Nature* **363**: 133–140.
- Hedgecock, E.M., J.G. Culotti, J.N. Thomson, and L.A. Perkins. 1985. Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. *Dev. Biol.* **111**: 1153–1157.
- Hill, R.J. and P.W. Sternberg. 1992. The *lin-3* gene encodes an inductive signal for vulval development in *C. elegans*. *Nature* **358**: 470–476.
- Horvitz, H.R. and P.W. Sternberg. 1991. Multiple intercellular signalling systems control the development of the *C. elegans* vulva. *Nature* **351**: 535–541.
- Horvitz, H.R. and J.E. Sulston. 1980. Isolation and genetic characterization of cell-lineage mutants of the nematode *Caenorhabditis elegans*. *Genetics* **96**: 435–454.
- Keen, J.H. 1987. Clathrin assembly proteins: Affinity purification and a model for coat assembly. *J. Cell Biol.* **105**: 1989–1998.
- . 1990. Clathrin and associated assembly and disassembly proteins. *Annu. Rev. Biochem.* **59**: 415–438.
- Keen, J.H., M.H. Chesnut, and K.A. Beck. 1987. The clathrin coat assembly polypeptide complex: Autophosphorylation and assembly activities. *J. Biol. Chem.* **262**: 12182–12188.
- Kirchhausen, T., K.L. Nathanson, W. Matsui, A. Vaisberg, and E.P. Chow. 1989. Structural and functional division into 2 domains of the large (100-kD to 115-kD) chains of the clathrin-associated protein complex AP-2. *Proc. Natl. Acad. Sci.* **86**: 2612–2616.
- Kirchhausen, T., A.C. Davis, S. Frucht, B.O. Greco, and G.S. Payne. 1991. AP17 and AP19, the mammalian small chains of the clathrin-associated protein complexes show homology to YAP17p, their putative homolog in yeast. *J. Biol. Chem.* **266**: 1153–1157.
- Krause, M. and D. Hirsh. 1987. A trans-spliced leader sequence on actin mRNA in *Caenorhabditis elegans*. *Cell* **49**: 753–761.
- Kretz, K.A., G.S. Carson, and J.S. O'Brien. 1989. Direct sequencing from low-melting agarose with sequenase. *Nucleic Acids Res.* **17**: 5864.
- Matsui, W. and T. Kirchhausen. 1990. Stabilization of clathrin coats by the core of the clathrin-associated protein complex AP-2. *Biochemistry* **29**: 10791–10798.
- Mello, C.C., J.M. Kramer, D. Stinchcomb, and V. Ambros. 1991. Efficient gene transfer in *C. elegans* after microinjection of DNA into germline cytoplasm: Extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10**: 3959–3970.
- Nakayama, Y., M. Goebel, B.O. Greco, and S. Lemmon, E.P.C. Chow, and T. Kirchhausen. 1991. The medium chains of the mammalian clathrin-associated proteins have a homolog in yeast. *Eur. J. Biochem.* **202**: 569–574.
- Ochman, H., A.S. Gerber, and D.L. Hartl. 1988. Genetic applications of an inverse polymerase chain reaction. *Genetics* **120**: 621–623.
- Pearse, B.M.F. and M.S. Robinson. 1990. Clathrin, adapters, and sorting. *Annu. Rev. Cell Biol.* **6**: 151–171.
- Peeler, J.S., W.C. Donzell, and R.G.W. Anderson. 1993. The appendage domain of the AP-2 subunit is not required for assembly or invagination of clathrin-coated pits. *J. Cell. Biol.* **120**: 47–54.
- Ruvkun, G., V. Ambros, A. Coulson, R. Waterston, J. Sulston, and H.R. Horvitz. 1989. Molecular genetics of the *Caenorhabditis elegans* heterochronic gene *lin-14*. *Genetics* **121**: 501–516.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Seeger, M. and G.S. Payne. 1992. Selective and immediate effects of clathrin heavy-chain mutations on Golgi membrane-protein retention in *Saccharomyces cerevisiae*. *J. Cell. Biol.* **118**: 531–540.
- Schmid, S.L. 1992. The mechanism of receptor-mediated endocytosis—More questions than answers. *BioEssays* **14**: 589–596.
- Sorkin, A., and G. Carpenter. 1993. Interaction of activated EGF receptors with coated pit adaptins. *Science* **261**: 612–615.
- Sternberg, P. W. 1993. Intercellular signaling and signal transduction in *C. elegans*. *Annu. Rev. Genet.* **27**: 497–520.
- Thomas, J.H. 1990. Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics* **124**: 855–872.
- Thurieu, C., J. Brosius, C. Burne, P. Jolles, J. Keen, R.J. Mat-
taliano, E.P. Chow, K. Ramachandran, and T. Kirchhausen.

1988. Molecular cloning of complete amino acid sequence of AP50, an assembly protein associated with clathrin-coated vesicles. *DNA* **7**: 663–669.
- Waterston, R., C. Martin, M. Craxton, C. Huynh, A. Coulson, L. Hillier, R. Durbin, P. Green, R. Shownkeen, N. Halloran, M. Metzstein, T. Hawkins, R. Wilson, M. Berks, Z. Du, K. Thomas, J. Thierry-Mieg, and J. Sulston. 1992. A survey of expressed genes in *Caenorhabditis elegans*. *Nature Genet.* **1**: 114–123.
- Williams, B.D., B. Schrank, C. Huynh, R. Shownkeen, and R.H. Waterston. 1992. A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. *Genetics* **131**: 609–624.
- Zetka, M.C. and A.M. Rose. 1992. The meiotic behavior of an inversion in *Caenorhabditis elegans*. *Genetics* **131**: 321–332.

unc-101, a gene required for many aspects of *Caenorhabditis elegans* development and behavior, encodes a clathrin-associated protein.

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